(substrate and sodium ions in the core) were performed, in a fully atomistic, solvated bilayer environment. In the Apo_MD and the Na_MD simulations, the HP1 loop has a much higher mobility than the HP2 loop. However, the release of the substrate into the intracellular solvent in Asp_Na_MD, required the motions of both the HP1 and the HP2 loops. The opening up of HP2 loop facilitates solvation of the binding site resulting in the substrate being dislodged from its position. Prior to substrate release, the HP1 loop moves further down into the solvent, exposing the HP1-tip and the substrate to the solvent, followed by its subsequent release into the intracellular solvent. These results suggest that the intracellular gating involves sequential opening of both HP1 and HP2 loops.

1342-Pos Board B252
Ligand Exit and Entry Pathways for Monoamine Transporters
Bonnie A. Merchant, Jeffry D. Madura.
The monoamine transporters are targets for various medicinal and illegal drugs that affect mood and behavior. Of particular interest are the dopamine (DAT) and serotonin (SERT) transporters of which the three-dimensional structures are unknown. A three-dimensional structure homologous to DAT and SERT, both in sequence and in function, is the leucine transporter ($LeuT_{Aa}$). While there is significant binding and uptake data, some structural information and homology models, there is no clear understanding of the transport pathways for ligands of $LeuT_{Aa}$, DAT or SERT. The Random Acceleration Molecular Dynamics (RAMD) method as implemented in NAMD, was used to study the entry and exit pathways of various chemically relevant substrates in $LeuT_{Aa}$ and a homology model of DAT. Example pathways as illustrated in Figure 1. Free energy scores of the pathways have been characterized via the Multi-Configuration Thermodynamic Integration method. Several sites of low free energy score have been indentified, which correspond to primary and secondary substrate pockets of the transporters. Detailed free energy and structural results of the transport pathways will be presented.

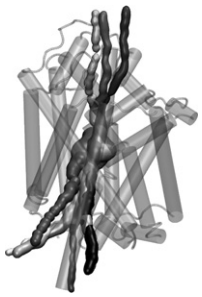

Figure 1. Representative transport pathways of leucine through LeuTAa using RAMD.

1343-Pos Board B253
Proton Transport and Conformational Changes in H+/CL- Exchangers
Mattia Malvezzi, Alessandra Picollo, Alessio Accardi.
CLC transporters localize to the membranes of intracellular compartments, such as lysosomes and endosomes, where they mediate a variety of physiological roles by exchanging 2 Cl^- ions for 1 H^+. Very little is known about the mechanisms underlying the transport process and many questions are still unanswered, in particular the H^+ pathway has not been identified and which conformational changes the transporters undergo is still not clear. To elucidate these important aspects we decided to investigate how protons translocate through the protein by studying the deuterium kinetic isotope effect and to probe the conformational changes by measuring the temperature dependence of the transport rate of the human transporter CLC-5 and the bacterial homologue CLC-ec1. We found that both CLC-5 and CLC-ec1 have similar thermodynamic profile. The transport rate in deuterium is decreased by ~20-40%, suggesting a H^+ movement through a hydrogen-bonded pathway, possibly formed by water. Both transporters also have a similar and modest temperature dependence, suggesting that the proteins undergo limited conformational changes. Interestingly, we observed unaltered apparent activation enthalpy of transport when Cl^- or H^+ binding, coupling or transporter gating are impaired, while the transport rates are affected. We hypothesize that H^+ movement through a CLC transporter takes place along a series of hydrogen bond formed by water molecules and that only limited conformational changes occur during the transport cycle. Finally, we propose a transport mechanism where several rate-limiting steps with similar apparent activation enthalpies are involved, instead of a single rate-limiting step mechanism.

1344-Pos Board B254
Analysis of the Oligomeric State of Surface-Localized Proton-Coupled Folate Transporter by Blue Native Polyacrylamide Gel Electrophoresis
Prachi Nakashe, Michaela Jansen.
Folate vitamins are essential for DNA replication and cellular proliferation. However, mammalian organisms are devoid of de novo folate biosynthesis and thus rely on dietary sources to meet their metabolic needs. The proton coupled folate transporter (PCFT/SLC46A1) has been recently identified as the molecular entity of the carrier mediated intestinal folate uptake pathway for folic acids from food sources. PCFT is also involved in the absorption of chemotherapeutically used antifolates. Currently, there is limited information about the structure and function of PCFT. Hydropathy analysis suggests that there are 10-12 transmembrane segments. Further, using the Substituted Cysteine Accessibility Method (SCAM) evidence was provided for a 12 transmembrane segment topology. Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) is a technique for separation of protein complexes in a native state with high resolution. We expressed PCFT in Xenopus laevis oocytes. Oocyte plasma membranes were polymerized to the vitelline membrane using ludox colloidal silica solution and polyacrylic acid, isolated by centrifugation, and plasma membrane proteins subsequently solubilized with digitonin and separated by BN-PAGE. The separation characteristics of native PCFT were compared to a molecular ruler produced by partial dissociation of homopentameric 5-hydroxytryptamine type 3A (5HT3A) receptors. Under native conditions, 5HT3A subunits largely migrated as a pentamer and PCFT only as a monomer. Treatment with denaturing agents generated a ladder of five bands for 5HT3A subunits, which consisted of monomer, dimer, trimer, tetramer and pentamer. Addition of crosslinking agents resulted in migration of 5HT3A subunits as a pentamer, even in the presence of denaturing agents. In contrast, crosslinking agents did not induce oligomeric assemblies of PCFT. These results indicate that functional plasma-membrane bound PCFT is a monomeric protein.

1345-Pos Board B255
Sets of Local Entropy-Enthalpy Change Leads to Global Entropy-Enthalpy Change in SERCA
Anu Nagarajan, Thomas B. Woolf.
Major conformational changes are involved in the multi-step catalytic cycle of the sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) pump. The movement and rotation of the Actuator (A) domain is crucial for ion translocation. The A-domain is connected to the trans-membrane helices through linker regions. Mutational studies on the A-M3 linker region show that varying the length of this region causes significant changes in the rate of the conformational transitions (JBC (2009),284,12258-12265). The focus of this research is to study the impact of these mutations on the structural changes during the transition from E1 to E2, the behavior of the A-M3 linker region, and the overall rate of the conformational transitions. In order to achieve faster computation of transitions, we implemented the MARTINI coarse-grained protein and lipid model in CHARMM. We used Dynamic Importance Sampling (DIMS) to compute transitions from the E1 to the E2 state for both directions of each mutant in both coarse-grained and all atom models. Analysis of the transitions across mutants shows that the angle formed by A-P-N domains changes by up to 20 degrees with an increasing number of inserts into the A-M3 linker region. Estimates of barrier crossing time from the simulation and experimental values are highly correlated (R^2=0.934). Quasiharmonic analysis on the domains, linkers and transmembrane helices show entropic changes between the mutants and compensation effects. Interaction energies of the same regions indicate entropic-enthalpic compensation. Further investigation of the end state simulations shows changes in the number of high density water sites around the A-M3 linker region across the mutants. The varying degree of the change in volume due to water sites across all the mutants indicates a ripple effect where the local entropy-enthaply changes translate to global entropy-enthalpy changes.

1346-Pos Board B256
Functional Analysis of Transmembrane Domain 3 in NKCC1
Suma Somasekharan, Biff Forbush.
We sought to determine the functional roles of residues in transmembrane domain (TM) 3 of human NKCC1 using tryptophan and cysteine scanning mutagenesis. We generated a structural alignment of the transmembrane domains of NKCC1 to the related APC transporters ApcT and AdiC, and obtained 3-D alignments using Modeller. Based on these alignments, residues 368 to 380 were predicted to form part of the inner 2/3 of the translocation pore. We substituted these residues with tryptophan and cysteine and determined the impact of the changes on protein synthesis and cell surface delivery by Western blotting and immunofluorescence microscopy. Most of our mutants expressed and localized similar to wild type NKCC1 and these were analyzed in depth for transporter function by means of Rb^{86} influx assays. A working hypothesis is that tryptophan mutants that are much reduced in function are too hydrophobic for the solvent interface and those residues that retain function are either protein interior or lipid facing. The pattern of the tryptophan scan followed an alpha helical periodicity. Based on the tryptophan scan we deduced that the non-functional mutants I368W, G369W, F372W, A375W, N376W, A379W are pore lining residues. Cysteine scanning complemented the results of the tryptophan scan. Since cysteine is a mild mutation, most mutants were functional. However I368C, G369C, A379C showed dramatic reduction or loss of function

which suggested that either the cysteine was too large or disrupted substrate binding. Also F372C and N376C residues showed reduced chloride and rubidium affinity. The combined results of the two scanning approaches are consistent with our predictions of TM3 being an alpha helical domain with I368, G369, F372, A375, N376, A379 being pore lining residues.

1347-Pos Board B257

Synergistic Substrate binding in the CLC-Ec1 Exchanger

Alessandra Picollo, Alessio Accardi.

Secondary active transporters couple the electrochemical gradients of two or more substrates to catalyze their stoichiometric exchange across biological membranes. In canonical models of alternating access exchange binding of different molecules is mutually exclusive or competitive. CLC-ec1 is a structurally known H^+/Cl^- exchanger of the CLC family and it has served as a guide in understanding the basic functional properties of CLC channels and transporters. Two glutamates (E148, E203) define the external and internal end points of the H^+ permeation pathway. However the coupling mechanism of these exchangers is still obscure. We used Isothermal Titration Calorimetry to probe the coupling between H^+/Cl^- binding and transport by measuring the enthalpy of Cl^- binding in different buffers. We found that binding of Cl^- and H^+ is synergistic: binding of 2 Cl^- to CLC-ec1 induces the binding of 1 H^+. Thus, the stoichiometries of binding and transport are equal but have opposite sign. Cl^- binding induces the uptake of 1 H^+ from the buffer in the E203Q mutant despite the fact that this mutant is unable to transport H^+. In contrast, Cl^- and H^+ binding become decoupled in the E148A mutant. Thus, while both glutamates are essential for coupling movement of H^+ and Cl^- only E148 controls their coupled binding while protonation of E203 takes place in a Cl^--independent manner. Decoupling H^+ and Cl^- fluxes by mutating residues that impair Cl^- binding leads to a parallel disruption of their binding synergism, strongly suggesting that this step is critical for the transport process.

In conclusion, our results show that during the exchange cycle binding of Cl^- at one side of the membrane induces binding of H^+ at the other side. This, implies that the transport cycle of CLC-ec1 is drastically different from conventional alternating access mechanisms.

1348-Pos Board B258

Identification of a Substrate Translocation Trajectory in the Inward-Facing Conformation of the Monocarboxylate/H+ Symporter Jen1

George Diallinas, Isabel Soares-Silva, Joana Sá Pessoa,
Vassilios Myrianthopoulos, Emmanuel Mikros Mikros, Margarida Casal.

Previous mutational analysis of Jen1p, a Saccharomyces cerevisiae monocarboxylate/H+ symporter of the Major Facilitator Superfamily, has suggested that the consensus sequence 379NXX[S/T]HX[S/T]QD387, located in transmembrane segment VII (TMS-VII), is part of the substrate translocation pathway. In this work, we rationally design and analyse novel mutations concerning residues in TMS-V and TMS-XI. Our analysis identifies several residues critical for Jen1p function. Among these, F270 (TMS-V) and Q498 (TMS-XI) function as specificity determinants for the distinction of mono- from di-carboxylates, whereas N501 is irreplaceable for function. Using a novel theoretical model created on the basis of Jen1p similarity with GlpT permease, we demonstrate that all polar residues in TMS-VII and TMS-XI, shown previously and herein to be critical for function and/or specificity (N379, H383, D387, Q498, N501), are perfectly aligned in a row along an imaginary axis that lies parallel to a protein pore. The model also predicts that the flexible side-chain of an additional polar residue, R188 in TMSII, faces the pore and subsequent mutational analysis showed that this aminoacid, similar to most polar residues of the pore, is irreplaceable for function. Finally, our model shows that the location of F270 and Q498 could justify their role in substrate specificity. Independent substrate docking approaches reveal a 'trajectory-like' displacement of the substrate within the Jen1p pore. In this inward-facing trajectory the flexible side-chain of R188 plays a major dynamic role mediating the orderly relocation of the substrate by subsequent H-bond interactions involving itself and residues H383, N501 and Q498.

1349-Pos Board B259

Molecular Mechanisms of Monovalent Cation Selectivity in Na+-Coupled Secondary Transporters from Free Energy Simulations with Implicit and Explicit Treatment of Electronic Effects

Bogdan Lev, Chunfeng Zhao, Sergei Noskov.

The ability to efficiently discriminate different cations is essential for the proper physiological functioning of channels and transporters. Strict monovalent selectivity of Na+-coupled secondary transporters is essential to realize the strong coupling between the transport of the substrate and Na+ flux. Loss of selectivity would result in the substrate being pumped outward. The x-ray structures of Na+-dependent amino-acid transporters (LeuT and Glt) [1,2], provides a great opportunity to better understand the molecular basis of monovalent cation selectivity.

Extensive all-atom free energy molecular dynamics simulations with hamiltonian construted with additive, polarizable force-fields and QM/MM descriptions of the binding pocket are performed at various occupancy states of the binding sites. In this work, we demonstrate that there is a collective effect of multiple binding sites on a total selectivity for Na+ over K+ (and Tl+) in LeuT and Glt transporters. The polarizable models of metal ions and protein (for sites Na1 and Na2 identified in structural studies) employed to provide better understanding for ion selectivity [3] as well as to mechanism of ion-substrate coupling in Glt transporter. The role of local connectivity, site rigidity and atomic polarization in monovalent cation selectivity is discussed. We will also present results from decomposition analysis.

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[2] Yamashita, A., Singh, S.K., Kawate, T., Jin, Y., Gouaux, E., 2005, Nature 437: 215-223.
[3] Lev B., Salohub D., Noskov S., Computational Life Sciences, Vol. 2, No. 1, pp. 12-20.

1350-Pos Board B260

The ATPase Activity of CFTR Measured in Living Cells

Anna Seelig, Matthias Zwick, Manuel Hellstern.

The cystic fibrosis transmembrane conductance regulator CFTR (ABCC7) is a homolog of the adenosine triphosphate (ATP) binding cassette (ABC) transporter P-glycoprotein (ABCB1) and functions as a chloride channel. Opening of the chloride channel requires ATP binding at the nucleotide binding domains and is enhanced by phosphorylation of the regulatory domain. Chloride flux is generally monitored by means of the patch clamp technique. Here we show that ATPase activity can be easily measured in living cells by monitoring the extracellular acidification rate (ECAR) using silicon chip technology as shown previously for P-glycoprotein. The ECAR of CFTR transfected Chinese hamster ovary (CHO) and the corresponding wild type cells, respectively, were measured under different phosphorylation conditions, using CPT-cAMP and forskloin. Whereas wild type cells showed only negligibly small effects, the ECAR of CFTR transfected cells increased at low concentrations, reached a maximum (up to 180% of the basal value), and decreased again at high concentrations, yielding bell-shaped activity curves. The lactate concentration was assessed in parallel, revealing that the extracellular acidification was due entirely to the extrusion of lactate which is a byproduct of anaerobic ATP synthesis. As ATP synthesis directly correlates with ATP hydrolysis the present approach allows a quantitative estimate of the rate ATP hydrolysis by CFTR under the different phosphorylation conditions.

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1351-Pos Board B261

Capturing Large-Scale Conformational Dynamics of P-Glycoprotein by MD Simulations

Po-Chao Wen, Emad Tajkhorshid.

P-glycoprotein is one of the most biomedically relevant ABC transporters, due to its frequent over-expression in cancer cells and involvement in their multidrug resistance. The crystal structure of murine P-glycoprotein has been reported in the nucleotide-free state. Employing molecular dynamics simulations we have investigated its conformation, dynamics and transport mechanism in a membrane environment. The simulation results show that, surprisingly, P-glycoprotein is capable of adopting an unusually wide range of conformations under the nucleotide-free conditions, highlighted by the degree of separation between the two nucleotide-binding domains (NBDs). During the equilibrium simulations, the distance between the two NBDs fluctuates over a range of at least 20 Å, exhibiting both wider and narrower NBD openings than what is captured in the crystal structure. Moreover, we have been able to successfully dock ATP and Mg^{2+} in the Walker A motif, and generate an ATP-bound state that remains stable throughout the following 50 ns simulations. Interestingly, the presence of ATP and Mg^{2+} does not significantly alter the distribution of P-glycoprotein between open and closed conformations during the simulations, implying that the closed dimeric NBDs might be an innate conformation already existing in the nucleotide-free form, and the presence of nucleotide only stabilizes such a closed form. These results are in very close agreement, and in fact rationalize the reported high fluctuations at the NBDs measured in several other ABC exporters, which is possibly representing a fundamental difference in the transport mechanisms between ABC exporters and importers.